Plant Physiol. (1996) 112: 585–590

Cool Temperature-Induced Chlorosis in Rice Plants

II. Effects of Cool Temperature on the Expression of Plastid-Encoded Genes during Shoot Growth in Darkness

Riichiro Yoshida*, Akira Kanno, and Toshiaki Kameya

Institute of Genetic Ecology, Tohoku University, Aoba-ku, Sendai 980–77, Japan

It has been proposed that cool temperature-induced chlorosis (CTIC) in *Indica* cultivars of rice (*Oryza sativa* L.) is caused by cell growth and plastid development being impeded at cool temperatures. Since it is well known that the overall rate of transcription of plastid-encoded genes changes dramatically during the early phases of plastid development, in this study we focused on the patterns of expression of these genes. Northern blot analysis revealed that the level of 16S rRNA is decreased in a CTIC-sensitive rice cultivar grown at a cool temperature. The expression of the gene for the β subunit of plasmid RNA polymerase (*rpoB*) was shown to be somewhat disturbed, particularly in terms of its resuppression under cool conditions. The level of transcripts or proteins of plastid-encoded photosynthetic genes was also decreased in a CTIC-sensitive cultivar at a cool temperature. These results suggest that the temperature-dependent inhibition of the onset of gene expression encoding the transcription/translation apparatus may be primarily involved in the mechanism causing CTIC.

CTIC in developing seedlings is one symptom of cold injury in rice plants (*Oryza sativa* L.), particularly in cultivars of the *Indica* type (Choung and Omura, 1982; Kaimori and Takahashi, 1985). When seedlings are exposed to cool weather, the newly emerging leaves lack pigments. Once such chlorotic leaves have developed, they remain white even at permissive temperatures without withering. To gain insight into the molecular mechanism of CTIC in rice plants, we previously established a model using dark-grown seedlings to mimic in the laboratory the CTIC that occurs in the field (Yoshida et al., 1996). In our model, the growth of immature leaves and the development of undifferentiated plastids to mature etioplasts in darkness was influenced by cool temperatures.

The expression of CTIC in SUR is bimodally dependent on temperature, suggesting a growth-dependent phenomenon (Yoshida et al., 1996). Biochemical and ultrastructural studies suggest that a reduction in the level of NADPH-protochlorophyllide oxidoreductase and arrested etioplast development might be closely linked to the expression of the CTIC phenotype.

In monocots, proplastids are converted into mature organelles during the development of mesophyll leaf cells from leaf primordial cells (Baumgatner et al., 1989). During the early phases of development the overall rate of transcription of plastid-encoded genes changes dramatically (Baumgartner et al., 1993). In particular, the rate of transcription and the level of transcripts of genes encoding the transcription/translation apparatus in plastids are dramatically elevated relative to most of the genes that encode the photosynthetic apparatus early in the development of organelles in synchrony with cell growth (Bisanz-Seyer et al., 1989; Baumgartner et al., 1993; Harrak et al., 1995). The rate of posttranscriptional processes, including RNA processing and stability, are also closely linked to the development of plastids (Klaff and Gruissem, 1991; Kim et al., 1993).

Considering these lines of evidence, in the present study we focused on the expression of plastid-encoded genes as a function of growth temperature, in particular, the genes that encode the transcription/translation apparatus. We chose several DNA probes for northern blot analysis, including probes for 16S rRNA, β subunits of plastid RNA polymerase (*rpoB*), and the *psbB* operon, and probes for genes that encode the photosynthetic apparatus, to monitor molecular events during the development of plastids. Western blot analysis was also performed to examine the accumulation of gene products.

Results presented here show that cool temperature-induced suppression of gene expression, particularly suppression of the genes that encode the transcription/translation apparatus, is a primary phenomenon in the mechanism of CTIC.

MATERIALS AND METHODS

The rice (*Oryza sativa* L.) cultivars SUR and O-195 were used as CTIC-sensitive and CTIC-tolerant materials, respectively.

Growth Conditions

Seeds of each cultivar were submerged in water at 25°C in darkness. After sterilization with 70% ethanol for 30 s and with 1% $HClO_3$ for 15 min, seeds were incubated on wet filter paper in Petri dishes in darkness at 25°C. Germi-

* Corresponding author; e-mail riichi@bansui.ige.tohoku.ac.jp; fax 181-22-263-9845.

Abbreviations: CF_1, coupling factor 1; CTIC, cool temperature-induced chlorosis; O-195, CTIC-resistant rice cultivar Ouu no. 195; SUR, CTIC-sensitive rice cultivar Surjamukhi.

nated seeds were transplanted to a medium that consisted of modified Hoagland solution plus 0.4% agar (Yoshida et al., 1996). Seedlings were allowed to grow in darkness at 25°C for 4 to 5 d or at 15°C for 14 to 16 d to an appropriate growth stage (about 6–7 cm in shoot length).

Extraction of Total RNA and RNA Gel-Blot Analysis

Five-day-old seedlings grown at 25°C and 14-d-old seedlings grown at 15°C were used for the extraction of total RNA. About 1 g of rice shoots was excised from seedlings under a green safelight and partially frozen in liquid nitrogen. These shoots were stored at −80°C prior to the extraction of RNA. Total RNA was isolated with a commercial kit (Extract-A-Plant RNA Isolation Kit, Clontech, Palo Alto, CA). The RNA was fractionated on a 1.5% formaldehyde agarose gel at a voltage of 30 V, then transferred to a nylon membrane (Magnacharge, Micron Separations, Westboro, MA). The membrane was prehybridized in hybridization buffer (5× SSC, 1% blocking reagent, 0.1% *N*-lauroylsarcosine, 0.02% SDS) at 65°C for 5 to 6 h. Hybridization was carried out for 15 to 16 h at 65°C in a buffer that contained a denatured, digoxigenin-labeled probe. After hybridization, the filter was washed twice in 2× SSC and 0.1% SDS for 5 min each, and then washed twice in 0.2× SSC and 0.1% SDS at 65°C for 15 min each. Nonradioactive digoxigenin-labeled probes were detected with a chemiluminescent substrate (CSPD, Boehringer Mannheim). The chemiluminescent signals were recorded on x-ray film (X-Omat, Kodak).

For northern analysis of plastid-encoded gene transcripts, subclones from a clone bank of rice ctDNA (Hirai et al., 1985) were used as probes. The probe for mitochondria *atpA* from pea was kindly provided by Dr. K. Nakamura (Morikami and Nakamura, 1987).

Preparation of Plastid-Enriched and Mitochondrion-Enriched Fractions

Plastid-enriched fractions were prepared from second leaves as reported previously (Yoshida et al., 1996). For preparation of mitochondrion-enriched fractions, the plastids were centrifuged at 2,500*g* and the resulting supernatant was again centrifuged at 4,500*g* for 10 min, and then at 10,000*g* for 10 min. The final pellet was designated the mitochondrion-enriched fraction.

Western Blots

Total protein from isolated plastids was fractionated by SDS-PAGE in a 12% (w/v) polyacrylamide gel and transferred to a PVDF membrane in a semidry blotting apparatus, as previously reported (Yoshida et al., 1996). Immunological detection was carried out with a western blotting analysis system (ECL System, Amersham), as previously reported (Yoshida et al., 1996).

The antisera specific to Rubisco from rice and to CF_1 and Cyt *f* from spinach were kindly provided by Dr. T. Mae (Makino et al., 1983) and Dr. J. Hidema (Hidema et al., 1991), respectively. The antiserum specific to subunit I of Cyt *c* oxidase from sweet potato was kindly provided by Dr. M. Maeshima (Nakagawa et al., 1987).

RESULTS

Alterations in Levels of Transcripts of Plastid-Encoded Genes

Rice seedlings were grown at 25 or 15°C in darkness until all had reached the same developmental stage, the second-leaf stage, and the same shoot length, 6 to 7 cm. In contrast to O-195, SUR exhibited typical CTIC in the second leaves after dark growth at 15°C. When SUR seedlings were grown under dark-warm conditions, most plastids were converted into fully developed etioplasts in parallel with the growth of mesophyll leaf cells from cells of leaf primordia, and were readily converted to chloroplasts upon subsequent illumination (Yoshida et al., 1996). Since CTIC in rice is closely associated with interference in plastid development during growth at cool temperatures, we focused on the patterns of expression of plastid-encoded genes, particularly genes that encode the transcription/translation apparatus, which are known to be transcribed at preferentially high rates early in plastid development (Baumgartner et al., 1993).

Total RNA was prepared from the second leaves, and plastid RNAs were quantified by northern hybridization. Figure 1A shows the patterns of expression of the gene for 16S rRNA and of the genes for β subunits of plastid RNA polymerase (*rpoB*). The accumulation of 16S rRNA was markedly suppressed in the CTIC-sensitive cultivar after growth at 15°C, as compared with the control (25°C). By contrast, 16S rRNA accumulated to nearly the same level in the CTIC-resistant cultivar as in the control grown under cool conditions.

Transcript levels of genes for subunits of plastid RNA polymerase are known to increase dramatically early in the development of plastids, and then to decline abruptly in parallel with the maturation of chloroplasts or etioplasts (Baumgartner et al., 1993). Gene transcript levels in rice seedlings reached a maximum after growth for 3 d under warm conditions, and then declined gradually (Fig. 1B). Therefore, we might expect that the transcript levels of *rpoB* might have already declined in control leaves grown at 25°C for 5 d because of the development of etioplasts to nearly the mature state. Indeed, only trace amounts of *rpoB* transcript levels were detected in the control leaves. By contrast, levels of 16S rRNA increased with time (Fig. 1B). High levels of the *rpoB* transcript continued to accumulate in leaves of SUR grown at a cool temperature, suggesting that the system for regulation of the expression of this gene (particularly its repression) is somewhat disturbed at the cool temperature (Fig. 1A). In O-195 *rpoB* transcript levels were also somewhat higher in leaves grown at a cool temperature than in the control leaves (Fig. 1A). However, the difference was not as pronounced as it was in the CTIC-sensitive cultivar.

Figure 2 shows the patterns of expression of *petB* and *psbB* (*petB* is transcribed as part of the *psbB* operon in rice) (Kanno and Hirai, 1993). The polycistronic precursors are

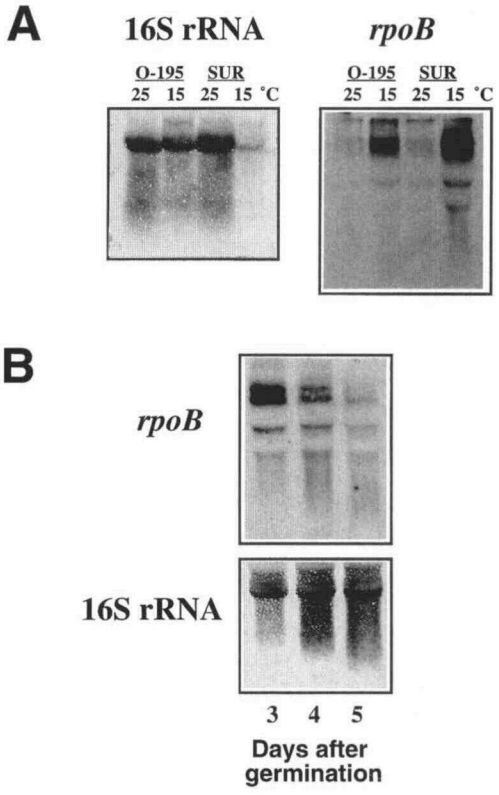

Figure 1. Nothern blot analysis of plastid-encoded 16S rRNA and *rpoB* expression. A, Effects of a cool growth temperature on the accumulation of 16S rRNA and *rpoB* transcripts in rice seedlings. B, Changes in the accumulation of *rpoB* and 16S rRNA transcripts in SUR 3 to 5 d after the beginning of germination in darkness at 25°C. Twenty micrograms of total RNA from rice seedlings grown in darkness was analyzed in each lane. Filters were probed with gene-specific probes derived from cloned rice ctDNA.

processed or spliced to yield monocistronic transcripts (Barkan, 1989; Barkan et al., 1994). Therefore, transcripts of several lengths might be expected during northern analysis with the specific probes. In fact, transcripts of several sizes were detected in both cultivars after growth in warm conditions, especially transcripts that hybridized with *petB* probes (Fig. 2). However, in the case of RNAs from SUR grown at a cool temperature, there was not only a reduction in the transcript levels, but the transcript pattern was also clearly altered. Although a transcript of more than 7.4 kb (Fig. 2, arrows) accumulated in leaves of SUR grown at 15°C, little of this transcript was detected in leaves from either cultivar grown at 25°C (Fig. 2). In leaves of SUR grown at 15°C, smaller transcripts of less than 1.6 kb were barely detectable.

Figure 3 shows the expression patterns of genes for proteins in the photosynthetic apparatus. *rbcL*, *atpA*, and *atpB* transcript levels were markedly decreased in the leaves of SUR grown at a cool temperature. O-195 transcript levels were also decreased to some extent at the cool temperature, but the signals were still clearly detectable. *atpA* mRNA is reportedly transcribed as part of a large polycistronic message in rice plants (Kanno and Hirai, 1993). In leaves of SUR grown at a cool temperature, the processing of the large polycistronic transcript is also significantly inhibited, as observed in the case of the *psbB* operon (Fig. 3).

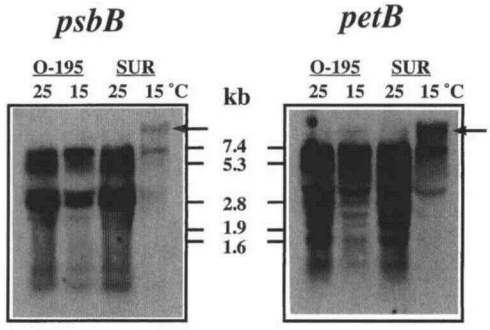

Figure 2. Effects of a cool growth temperature on the pattern of accumulation of *psbB* operon transcripts in rice seedlings. Twenty micrograms of total RNA from rice seedlings grown in darkness at 25 or 15°C was analyzed in each lane. Filters were probed with gene-specific probes derived from cloned rice ctDNA.

Reduction in the Accumulation of Proteins Encoded by the Plastid Genome

In view of the marked reduction in the transcript levels of genes for the translation apparatus (Fig. 1), the reduced accumulation of proteins encoded in the plastid genome was anticipated. Figure 4 shows the results of immunoblot analysis of the large subunit of Rubisco, the CF_1 complex of ATP synthetase, and Cyt *f* with their respective antisera. The protein level of the large subunit of Rubisco was reduced more than 6-fold in the plastid-enriched fraction from SUR as a result of growth at a cool temperature. Also, the nucleus-encoded small subunit of Rubisco was present at a reduced level in the plastid fraction (data not shown), and the accumulation of the CF_1 complex in SUR was prevented by cool temperature. Since CF_1 is predominately associated with prothylakoid membranes (Ryberg and Sundqvist, 1982), these results are consistent with the arrest of etioplast development at a cool temperature. The membrane protein Cyt *f* was also absent from the plastid-enriched fraction from SUR as a result of growth at a cool temperature.

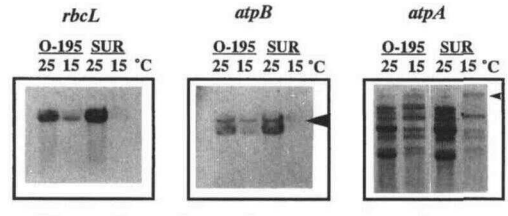

Figure 3. Effects of a cool growth temperature on the accumulation of plastid-encoded transcripts for the photosynthetic proteins in rice seedlings. Twenty micrograms of total RNA from rice seedlings grown in darkness at 25 or 15°C was analyzed in each lane. Filters were probed with gene-specific probes derived from cloned rice ctDNA for *rbcL*, *atpB*, and *atpA*.

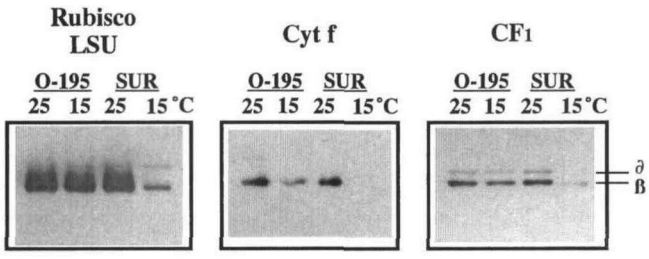

Figure 4. Effects of a cool growth temperature on the accumulation of plastid proteins. Plastid-enriched fractions were prepared from the second leaves of rice seedlings grown in darkness at 25 or 15°C, and 3 μg of protein was fractionated in each lane by SDS-PAGE. Gels were blotted onto a PVDF membrane and immunostained with antisera specific to Rubisco from rice, and to CF_1 and Cyt *f* from spinach.

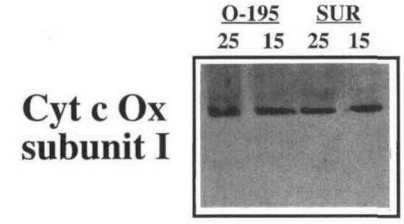

Figure 6. Effects of a cool growth temperature on the accumulation of subunit I of mitochondrial Cyt *c* oxidase. Mitochondrion-enriched fractions were prepared from the second leaves of rice seedlings grown in the darkness at 25 or 15°C, and 5 μg of protein was fractionated in each lane by SDS-PAGE. Gels were blotted onto a PVDF membrane and immunostained with antiserum specific to subunit I of Cyt *c* oxidase from sweet potato.

Effects of Temperature on the Expression of Mitochondrion-Encoded Genes

The development of plastids requires energy, which is mostly supplied by respiration, especially during growth in darkness (Roussell et al., 1991; Martinez-Zapeter et al., 1992). Therefore, mitochondrial functions, including respiration, must be under normal control to support the development of plastids. Figure 5 shows the transcript patterns revealed by northern blot analysis using cDNAs for 18S rRNA, *atpA*, and *cob* as probes. Regardless of the cultivar and the growth temperature, the genes were all expressed at a steady-state level. In contrast to the plastid-encoded 16S rRNA, accumulation of the mitochondrion-encoded 18S rRNA was totally unaffected by cool temperature. Subunit I of Cyt *c* oxidase, which is encoded in the mitochondrial genome, accumulated normally in the mitochondrion-enriched fraction isolated from leaves of SUR that had been grown in the darkness at 15°C (Fig. 6).

DISCUSSION

During the growth of rice seedlings in darkness, proplastids develop into mature etioplasts in synchrony with increasing cell age and/or cell growth, with a gradient of development from the basal leaf meristem to the tip of the leaf (Baumgartner et al., 1989). Rice cultivars of the *Indica* type, such as SUR, display CTIC when seedlings are grown within a certain critical range of cool temperatures. We reported previously that CTIC in SUR is closely associated with a disturbance in the development of etioplasts, which is primarily caused by growth at a cool temperature (Yoshida et al., 1996). It has been widely accepted that, during the early phases of plastid development, the differential expression of plastid-encoded genes for the transcription/translation apparatus occurs relative to the expression of genes for proteins of the photosynthetic apparatus (Bisanz-Seyer et al., 1989; Baumgartner et al., 1993; Harrak et al., 1995). We chose several probes for genes on the plastid genome, including those for 16S rRNA, the *rpoB*, the *psbB* operon, and other proteins of the photosynthetic apparatus, to examine the molecular events that occur in association with the development of etioplasts during growth in darkness at different temperatures.

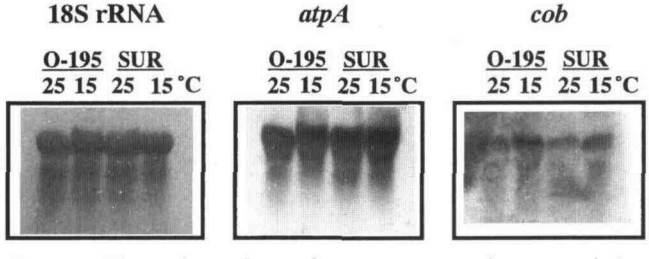

Figure 5. Effects of a cool growth temperature on the accumulation of mitochondrial transcripts. Twenty micrograms of total RNA from rice seedlings grown in darkness at 25 or 15°C was analyzed in each lane. Filters were probed with gene-specific probes derived from a cloned rice gene for 18S rRNA and the *atpA* and *cob* genes in pea mtDNA.

One of the most interesting findings in the present study was that the accumulation of 16S rRNA was specifically reduced in the CTIC-sensitive cultivar as a result of growth under cool conditions. This reduction might have been due either to a reduction in the transcriptional activity or to a defect in processing activity (Barkan, 1993; Harrak et al., 1995). However, we failed to find any unprocessed premature form of 16S rRNA in the cool-grown CTIC-sensitive cultivar (Fig. 1A). The 16S rRNA promoter contains prokaryotic −10 and −35 elements (Sun et al., 1986; Baeza et al., 1991) and therefore, it should be transcribed by a plastid-encoded RNA polymerase. According to Rajasekhar et al. (1991), the highly purified RNA polymerase from pea chloroplasts can transcribe the 16S rRNA and other mRNAs in vitro. The chloroplast RNA polymerase was shown to bind specifically to the promoter region of chloroplast 16S rRNA (as visualized by electron microscopy), a result that suggests that the plastid-encoded RNA polymerase is given high priority for transcription of 16S rRNA at the early phases of plastid development. It is possible, therefore, that the cool temperature-induced reduction in 16S rRNA levels in SUR might be due to a reduction in the rate of translation of the plastid-encoded genes for RNA polymerase.

It has been postulated that the expression of the *rpoB* operon at early phases of plastid development might be related to enhanced expression of a putative nucleus-encoded, plastid-localized RNA polymerase (Greenberg et

al., 1984; Rajasekhar et al., 1991; Hess et al., 1993; Lerbs-Mache, 1993). This hypothesis is based on several lines of evidence. Preliminary characterization of the *rpoB* promoter failed to reveal the presence of the −35 sequence element (Mullet, 1993). Furthermore, it was reported that mRNA for *rpoB-rpoC1-rpoC2* accumulates in plastids of *albostrains*, a barley mutant that lacks ribosomes (Hess et al., 1993), suggesting that the putative nucleus-encoded, plastid-localized RNA polymerase can transcribe the *rpoB* operon. In the present study, the *rpoB* in SUR continued to be expressed at high levels during growth at a cool temperature, whereas transcript levels had already declined in both cultivars prior to the time that seedlings reached the second-leaf stage under warm conditions. Therefore, the transcription/translation of the nucleus-encoded RNA polymerase and the localization of the enzyme in the plastid, possibly as a first signal for the initiation of plastid development (Mullet, 1993), can occur normally in SUR, even at a cool temperature. However, in the present study, we obtained no direct evidence for the translation of *rpo* mRNA in plastids of plants grown at the cool temperature. The observed general decline in expression levels of the *psbB* operon and genes for photosynthetic proteins supports the hypothesis that the translation of *rpoB* transcripts is inhibited at cool temperatures in SUR. For the gene expression necessary for a dramatic increase in the number of plastid ribosomes, small numbers of ribosomes already present in the plastids may participate in the initial step of translation (Harrak et al., 1995). However, it is unclear whether the pre-existing translation apparatus can function normally in SUR at a cool temperature. This important question needs to be answered in future studies.

Northern analysis of the *psbB* operon revealed that some regulatory reaction associated with the processing of the primary transcript did not occur efficiently in cool-grown SUR. Plastid-localized RNA-binding proteins might be involved in the process (Schuster and Gruissem, 1991). The longest transcript (more than 7.4 kb) in cool-grown SUR can be explained by two possible hypotheses: the promoter region for transcription of the *psbB* operon might be located far upstream in the 5′ direction of the *psbB* operon, or the site for the termination of transcription might be located farther downstream of *rpoA* (Kanno and Hirai, 1993).

The transcript of the *rpoB* operon in SUR continued to be expressed at high levels at the cool temperature, even though shoots had reached the same growth stage (the second-leaf stage) and the same size as the shoots grown at a warm temperature, an indication that the development of etioplasts had been arrested at an early phase. Although no evidence has been presented to date, some factors related to the developmental status of plastids might be involved in a feedback mechanism for repression of gene expression. The presence of numerous ribosomes or the completion of the synthesis of numerous ribosomes might be responsible for producing the putative factors involved in such a feedback mechanism. This hypothesis should be examined further to gain insight into the molecular mechanism of CTIC.

The energy required for plastid development during growth in darkness is supplied mainly by mitochondria. We found no indications of cool temperature-induced disturbances in mitochondrial function, even in SUR. Since no remarkable difference in the rate of shoot growth was observed between resistant and sensitive cultivars at a cool temperature (Yoshida et al., 1996), the respiratory functions needed for growth must have been operational. Thus, CTIC seems to be due primarily to impeded expression of plastid-encoded genes, particularly those for the transcription/translation apparatus.

As reported previously (Yoshida et al., 1996), the induction of chlorosis in SUR is dependent on the duration of exposure to cool temperatures (between 15 and 17°C). CTIC never occurs within 4 d of growth at 15°C, suggesting that the induction of the phenomenon might be reversible. However, if the growth phase proceeds beyond this critical period, a great disparity develops between cell growth and plastid development, leading to irreversible CTIC. Further study is needed to determine how the nucleus and plastids act in a coordinated manner in the transformation of the plastids.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Atsushi Hirai (Tokyo University, Japan) for the gift of a mitochondrial *cob* probe, and to Dr. Kenzo Nakamura (Nagoya University, Japan) for the pea mitochondrial 18S rRNA probe.

Received April 15, 1996; accepted June 21, 1996.
Copyright Clearance Center: 0032-0889/96/112/0585/06.

LITERATURE CITED

Baeza L, Bertrand A, Mache R, Lerbs-Mache S (1991) Characterization of a protein-binding sequence in the promoter region of the 16S rRNA gene of the spinach chloroplast genome. Nucleic Acids Res **19:** 3577–3581

Barkan A (1989) Tissue-dependent plastid RNA splicing in maize: transcripts from four plastid genes are predominantly unspliced in leaf meristems and roots. Plant Cell **1:** 437–445

Barkan A (1993) Nuclear mutants of maize with defects in chloroplast polysome assembly have altered chloroplast RNA metabolism. Plant Cell **5:** 389–402

Barkan A, Walker M, Nolasco M, Johnson D (1994) A nuclear mutation in maize blocks the processing and translation of several chloroplast mRNAs and provides evidence for the differential translation of alternative mRNA forms. EMBO J **13:** 3170–3181

Baumgartner BJ, Rapp JC, Mullet JE (1989) Plastid transcription activity and DNA copy number increase early in barley chloroplast development. Plant Physiol **89:** 1011–1018

Baumgartner BJ, Rapp JC, Mullet JE (1993) Plastid genes encoding the transcription/translation apparatus are differentially transcribed early in barley (*Hordeum vulgare*) chloroplast development. Plant Physiol **101:** 781–791

Bisanz-Seyer C, Li Y-F, Seyer P, Mache R (1989) The components of the plastid ribosome are not accumulated synchronously during early development of spinach plants. Plant Mol Biol **12:** 201–211

Choung PV, Omura T (1982) Studies on the chlorosis expressed under low temperature in rice, *Oryza sativa* L. *In* Bulletin of the Institute for Tropical Agriculture, Vol 5. Kyushu University, Fukuoka, Japan, pp 1–58

Greenberg BM, Narita JO, Deluca-Flaherty C, Gruissem W, Rushlow KA, Hallick RB (1984) Evidence for two RNA polymerase activities in *Euglena gracilis* chloroplasts. J Biol Chem **259:** 14880–14880

Harrak H, Lagrange T, Bisanz-Seyer C, Lerbs-Mache S, Mache R (1995) The expression of nuclear genes encoding plastid ribosomal proteins precedes the expression of chloroplast genes during early phases of chloroplast development. Plant Physiol **108:** 685–692

Hess WR, Prombona A, Fielder B, Subramanian AR, Borner T (1993) Chloroplast *rps*15 and the *rpo*B/C1/C2 gene cluster are strongly transcribed in ribosome-deficient plastids: evidence for a functioning non-chloroplast-encoded RNA polymerase. EMBO J **12:** 563–571

Hidema J, Makino A, Mae T, Ojima K (1991) Photosynthetic characteristics of rice leaves aged under different irradiances from full expansion through senescence. Plant Physiol **97:** 1287–1293

Hirai A, Ishibashi T, Morikami A, Iwatsuki N, Shinozaki K, Sugiura M (1985) Rice chloroplast DNA: a physical map and the location of the genes for the large subunit of ribulose 1,5-bisphosphate carboxylase and 32 kD photosystem II reaction center protein. Theor Appl Genet **70:** 117–122

Kaimori N, Takahashi N (1985) Expression of chlorosis at low temperature in rice plants. Society for the Advancement of Breeding Researches in Asia and Oceania Journal **17:** 57–66

Kanno A, Hirai A (1993) A transcription map of the chloroplast genome from rice (*Oryza sativa*). Curr Genet **23:** 166–174

Kim M, Christopher DA, Mullet JE (1993) Direct evidence for selective modulation of *psbA, rpoA, rbcL* and 16S RNA stability during barley chloroplast development. Plant Mol Biol **22:** 447–463

Klaff P, Gruissem W (1991) Changes in chloroplast mRNA stability during leaf development. Plant Cell **3:** 517–529

Lerbs-Mache S (1993) The 110-kDa polypeptide of spinach plastid DNA-dependent RNA polymerase: single-subunit enzyme or catalytic core of multimeric enzyme complexes? Proc Natl Acad Sci USA **90:** 5509–5513

Makino A, Mae T, Ohira K (1983) Photosynthesis and ribulose 1,5-bisphosphate carboxylase in rice leaves. Changes in photosynthesis and enzymes involved in carbon assimilation from leaf development through senescence. Plant Physiol **73:** 1002–1007

Martinez-Zapater JM, Gill P, Capel J, Somerville CR (1992) Mutations at the *Arabidopsis* CHM locus promote rearrangements of the mitochondrial genome. Plant Cell **4:** 889–899

Morikami A, Nakamura K (1987) Structure and expression of pea mitochondrial F_1 ATPase alpha-subunit gene and its pseudogene involved in homologous recombination. J Biochem (Tokyo) **101:** 967–976

Mullet JE (1993) Dynamic regulation of chloroplast transcription. Plant Physiol **103:** 309–313

Nakagawa T, Maeshima M, Muto H, Kajiura H, Hattori H, Asahi T (1987) Separation, amino-terminal sequence and cell-free synthesis of the smallest subunit of sweet potato cytochrome c oxidase. Eur J Biochem **165:** 303–307

Rajasekhar VK, Sun E, Meeker R, Wu B-W, Tewari KK (1991) Highly purified pea chloroplast RNA polymerase transcribes both rRNA and mRNA genes. Eur J Biochem **195:** 215–228

Roussell D, Thompson D, Pallardy S, Miles D, Newton K (1991) Chloroplast structure and function is altered in NCS2 maize mitochondrial mutant. Plant Physiol **96:** 232–238

Ryberg M, Sundqvist C (1982) Characterization of prolamellar bodies and prothylakoids fractionated from wheat etioplasts. Physiol Plant **56:** 125–132

Schuster G, Gruissem W (1991) Chloroplast mRNA 3′ end processing requires a nuclear-encoded RNA-binding protein. EMBO J **10:** 1493–1502

Sun E, Shapiro DR, Wu BW, Tewari KK (1986) Specific *in vitro* transcription of 16S rRNA gene by pea chloroplast RNA polymerase. Plant Mol Biol **6:** 429–439

Yoshida R, Kanno A, Sato T, Kameya T (1996) Cool temperature-induced chlorosis in rice plants. I. Relationship between the induction and a disturbance of etioplast development. Plant Physiol **110:** 997–1005